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14. ABSTRACT

There is an urgent need to develop both new approaches to the treatment of prostate cancer. Analysis of human prostate samples demonstrates that a specific signaling pathway, the Pim kinase pathway is elevated in the fibroblasts from human prostate tumors. To understand the role of myofibroblast/cancer associated fibroblasts (CAFs) in transformation, the laboratory proposes (1) to examine in detail the proteins secreted by the stroma that can modulate epithelial growth, (2) to evaluate the ability of Pim inhibitors to block this activity, and (3) to investigate whether exosomes can potentially be used as a biomarker of Pim kinase inhibitor activity. Results to date demonstrate that Pim increases in prostate stromal cells enhances protein synthesis, the levels of important transcription factors, long non-coding RNAs, and tyrosine kinases associated with signal transduction as well increased exosomal transfer both in cells co-cultured and when conditioned media is placed on prostate epithelial cells. These changes are blocked by the addition of Pim inhibitors. These results suggest that the Pim protein kinase can regulate stromal cell biology to modulate epithelial growth and that inhibitors of this protein kinase have the potential to block this process and thus inhibit tumor growth.

15. SUBJECT TERMS

Cancer associate fibroblasts (CAFs); Myofibroblasts; Pim protein kinase; Exosomes; Prostate stem cells, Pim inhibitors, AZD 1208 and LGH447

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Introduction
Prostate cancer (PCa) stromal cells, also known as myofibroblasts associated fibroblasts (CAFs) have a critically important interdependent interaction with the surrounding epithelial cells. Proteins secreted from CAFs stimulate PCa progression and metastasis. Interrupting this interdependency by targeting signal transduction pathways that mediate the production and secretion of these proteins is a novel approach to blocking PCa growth. In this proposal, the applicant research team demonstrates that protein kinases can induce normal prostate stromal fibroblasts to produce proteins that enhance PCa growth, thus mimicking a CAF-like phenotype. The addition of kinase inhibitory drugs that have entered Phase I clinical trials reverses this secretion. New results also demonstrate that changes in the proteins secreted by myofibroblasts can be detected in the urine of cancer patients, suggesting that these proteins can be used as a marker of the activity of drugs targeted at CAFs. *The knowledge gained through the analysis of CAFs from cancer patients proposed in this application is essential for the further development of new biomarkers that reflect stromal protein production*

Keywords
Cancer associated fibroblasts (CAFs)
Myofibroblasts
Pim Protein Kinases
Exosomes
Prostate Stem Cells
Pim Inhibitors, AZD1208 and LGH447

Accomplishments
Major goals of the project

1- Compare the secretome of immortalized prostate fibroblasts with freshly isolated human CAFs: develop a data set of proteins that are regulated by the Pim protein kinase in CAFs.
2- Examine whether inhibitors of PIM protein kinase block the tumor stimulating activity of myofibroblast/CAF-induced, including migration, invasion, and growth of epithelial tumor cells.
3- Investigate whether exosomes can potentially be used as a biomarker of Pim kinase inhibitor activity in myofibroblast/CAFs: document whether induced changes are reflected in EPS-urine of different Gleason grades of cancer.

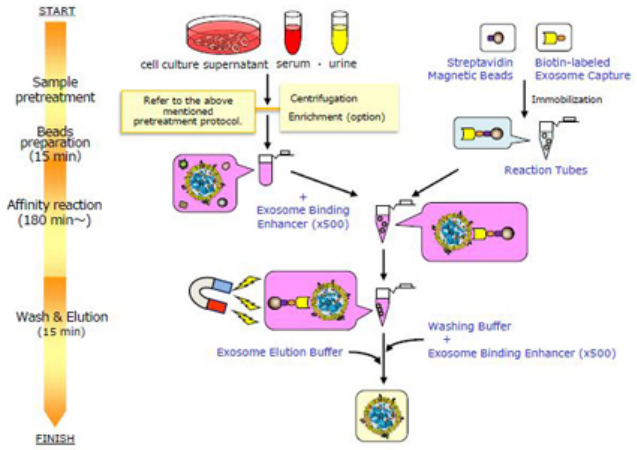
Accomplishments under these goals.

Major Task
Accomplishments during the reporting period

Task 1- Compare the secretome of normal prostate fibroblasts with inducible Pim1 with freshly isolated human CAFs: develop a data set of proteins that are regulated by the Pim1 protein kinase in prostate fibroblasts. To be able to isolate purified exosomes with confidence that these vesicles are pure and not contaminated by cellular debris. These purified vesicles will allow an analysis of the ability of Pim protein kinase to change and regulate the secretory pattern. Although some laboratories simply do a high speed spin to pellet vesicles, experiments by this team have found that these exosomes are not pure. To tackle this problem the following procedure has been developed. A prostate stromal cell line was derived that contained a Doxycyclin-inducible Pim 1 gene. BHPrS1-Tripz-Pim1. BHPrS1-Tripz-Pim1 stromal cell lines, typically, 20-40 $\times 10^6$ cells, were cultured in 25 mL DMEM medium with 5% exosome depleted FBS (Thermofisher). Using this FBS is critical to the success of this procedure. Culture supernatants were then harvested, and serially centrifuged to remove cells and debris (10 minutes at 300g, followed by 30 minutes at 3000g), and filtered

(0.22 µm) to remove small debris and microvesicles. Importantly experiments demonstrated that this media contained vesicles of multiple sizes and types. To enhance the purity of this preparation 150 mL of filtered media was concentrated to 1ml with the Centricon Plus-70 Centrifugal Filter (Ultracel-PL Membrane, 100 kDa) device using an Allegra® X-15R centrifuge at 3,500 g at 4°C. The concentrate was then recovered with a reverse spin at 1,000 g for 2 minutes. Exosome pellets were then isolated and concentrated using ultracentrifugation (3h at 110 000g, 4°C). Exosome pellets were suspended in PBS to a desired concentration and filtered (0.45 µm). The presence of Exosomes was validated using western blots to demonstrate enrichment of exosome marker proteins TSG101, CD63, and Flotillin1 known exosome proteins, and the absence of golgi vesicles and demonstrated by the absence of golgi protein GM130. *Figure 2* also shows exosomes isolated from doxycycline-treated Pim induced as well. Interestingly the Pim induced cells exhibited an increased level of the exosome marker CD63 compared with untreated controls suggesting that the number of exosomes was increased by Pim induction in stromal cells as defined by the proteins that were identified (see Figure 3 below). To compare the number of exosomes secreted by a fixed number of cells an Elisa kit measuring the acetylcholine esterase activity was used to quantify the number of exosomes per group. *Our results* that the number of exosomes produced by Pim expressing cells was increased and that this increase was inhibited by treating BHPrS1 cells with a Pim inhibitor (LGH447 3uM) (data not shown). This data demonstrates that the team is ready to attempt to identify proteins that are excreted as a result of Pim kinase overexpression. Importantly, it also shows that Pim kinase expression in stromal cells is increasing the quantity of exosomes released by these stromal cells. This finding is consistent with the hypothesis that Pim is playing an important role in stromal biology.

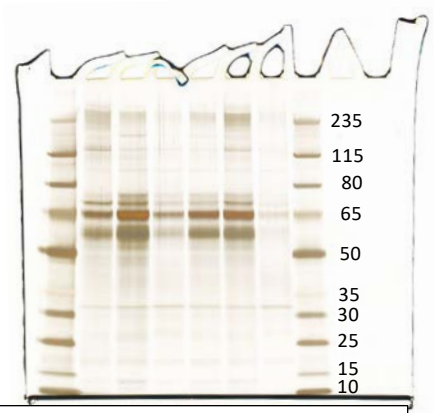
Figure 1. Exosome isolation protocol from Magcapture



Detailed Protocol for Isolation of Exosomes

Figure 2: Gel image of 1 ug protein of each sample loaded for Mass spec

Lane	sample	ug
1	MW marker	
2	Control BHPrS1-XO-1	1
3	Control BHPrS1-XO-2	1
4	Control BHPrS1-XO-3	1
5	Pim BHPrS1-XO-1	1
6	Pim BHPrS1-XO-2	1
7	Pim BHPrS1-XO-3	1
8	MW marker	
9	LB	



Exosomes were isolated from control BHPrS1prostate stromal cells or Dox-induced Pim expressing BHPrS1 cells and 1 ug of each prep was loaded SDS gels which were Comassie blue stained.

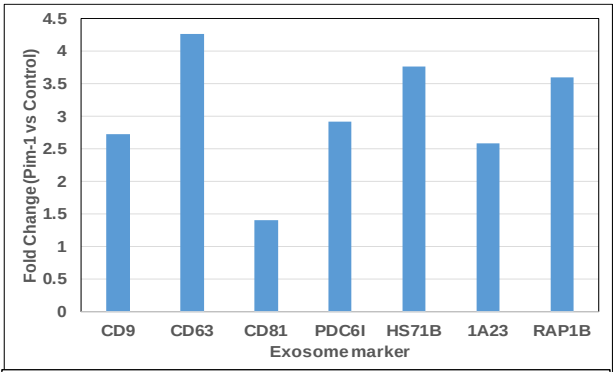
Mass spec description: Samples were sent for digestion and Sequential window acquisition of all theoretical mass spectra (SWATH) quantitation

Sample description: Exosomes isolated by WAKO MagCapture Kit from Human Prostate Stromal Cells

Purpose: Digestion and nanoLC-MS-SWATH quantitation of proteins across the triplicates samples

Protocol: Based on the protein concentrations provided, 10 ug was removed from each sample, concentrated by speedVac, reconstituted in Laemmli sample buffer and run on a mini gel with prestained markers until the dye front reached about 2 cm. The entire section of gel for each samples was excised (see gel images below),

Figure 3: Enrichment of exosomes in BHPrS1 with Pim induction



The level of markers found in exosomes isolated from Pim expressing BHPrS1 prostate stromal cells were compared to wild type cells.

and subjected to in gel digestion with trypsin after DDT reduction and iodoacetamide alkylation. The recovered peptide were reconstituted to 400 ng/uL in 0.1% formic acid such that 5 uL injected would equate to about 2 ug (the maximum amount for the nanoLC-MSMS trap column). The spectral libraries were generated from 3 sample combinations as follows: 1) equal mix of the 3 control samples, 2) equal mix of the 3 Pim samples, 3) equal mix of all samples. Subsequently, each control and each Pim sample was collected independently using a SWATH acquistion on a Sciex 5600 + quadrupole-tof system. The SWATH range was 350-750m/z with 8Da windows with a 1Da overlap. Data were collected at 50 ms with a cycle time of 3.15 sec. Gradient elution was from from 5% ACN to 40% in 70min (0.5%/min) from a 75um X 150 cm Thermo Acclaim c18 column. The spectral library and the SWATH analysis were done using Sciex Peakview software with the SWATH module. Protein areas were used to nomalize each samples using Total Area Normalization in Excel and the significance of protein changes between the control and Pim groups was done by T-Test.

2. SWATH Spectra Library--122 proteins were identified at a 1% protein FDR
a. The low number of protein may be related to a lower than expected protein load as judged by TIC of the TOF-MS which appears to be more consistent with about 0.5 ug load than the expected 2 ug.
b. The variability in protein load among the samples is also evident from the TIC profiles of both the control 3 and Pim 3 samples.
c. This low number of protein and variability in protein load has implications for sample normalization.

3. SWATH protein identification and comparative quantitation
   a) There were a number of exosome marker protein detected (Figure 2), suggesting a good enrichment of exosomes.
   b) The upregulated proteins on Pim-1 induction in Dox inducible BHPrS1 stromal cells are categorized in Table 1 and Figure 3.

Next Steps: The low amount analyzed and the variable levels of protein likely limited the number of protein detected and the ability to obtain relative quantitation data. One option might be to boast the amount of the control 3 and Pim 3 samples to get a closer load to the other 4 samples, but this may not add much since the spectral library is only 122 proteins and many of these are based on only one peptide detected. As such, the only real option is to expand the spectral library by first running about 4X more for each of the protein mixtures. This unfortunately would require preparing more samples for digestion. Since sample Pim-3 was only reported to 15 ug total (and we used 10 already) this may require more sample.

Results from First analysis:

Protein	Description	T-test	Fold change (PIM/C)
K2C1	Keratin, type II cytoskeletal 1 Present in intermediate filament	0.043395	3.285346
K1C9	Cytokeratin-9	0.087222	5.308301
K1C14	Cytokeratin-14	0.305837	1.594855
FINC	This gene encodes fibronectin, a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in extracellular matrix. The encoded preproprotein is proteolytically processed to generate the mature protein. Fibronectin is involved in cell adhesion and migration processes	0.590513	1.493957

	including embryogenesis, wound healing, blood coagulation, host defense, and metastasis.		
LAMA4	Laminin alpha 4 : Laminins, a family of extracellular matrix glycoproteins, are the major noncollagenous constituent of basement membranes. They have been implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis.	0.109969	1.61176
LAMB1	laminin subunit beta 1	0.176344	1.532355
SVEP1	Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1, May play a role in the cell attachment process.	0.575089	1.792747
ITGA2	This gene encodes the alpha subunit of a transmembrane receptor for collagens and related proteins. The encoded protein forms a heterodimer with a beta subunit and mediates the adhesion of platelets and other cell types to the extracellular matrix. Has a role in Bone metastasis	0.274516	1.750415
ITGB1	Integrins are heterodimeric proteins made up of alpha and beta subunits. At least 18 alpha and 8 beta subunits have been described in mammals. Integrin family members are membrane receptors involved in cell adhesion and recognition in a variety of processes including embryogenesis, hemostasis, tissue repair, immune response and metastatic diffusion of tumor cells. This gene encodes a beta subunit.	0.078302	1.421418
CD44	The protein encoded by this gene is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. It is a receptor for hyaluronic acid (HA) and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). This protein participates in a wide variety of cellular functions including tumor metastasis.	0.00626	1.842827
RAP1B	member of RAS oncogene family : This gene encodes a member of the RAS-like small GTP-binding protein superfamily. Members of this family regulate multiple cellular processes including cell adhesion and growth and differentiation. This protein localizes to cellular membranes and has been shown to regulate integrin-mediated cell signaling.	0.001455	1.707018
RAB10	member RAS oncogene family : RAB10 belongs to the RAS superfamily of small GTPases. RAB proteins localize to exocytic and endocytic compartments and regulate intracellular vesicle trafficking	0.012159	1.772537
CO1A1	Collagen type 1a1 and 1a2 are significantly higher in CAFs	0.572933	1.468351
CO1A2		0.635968	1.538359
EHD2	EH domain containing protein 2 : The encoded protein interacts with the actin cytoskeleton through an N-terminal domain and also binds to an EH domain-binding protein through the C-terminal EH domain. This interaction appears to connect clathrin-dependent endocytosis to actin, suggesting that this gene product participates in the endocytic pathway.	0.064491	1.500703
Microtubule-associated protein 1 light chain 3 alpha	MAP1A are microtubule-associated proteins which mediate the physical interactions between microtubules and components of the cytoskeleton. It may be involved in carcinogenesis.	0.019645	1.667629

STOM	Erythrocyte band 7 integral membrane protein : Data indicate that a stomatin-specific, raft-based process is involved in storage-associated vesiculation.	0.038822	2.000507
1433Z	14-3-3 protein zeta/delta : Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	0.064844	1.905192
MMP1	This gene encodes a member of the peptidase M10 family of matrix metalloproteinases (MMPs). Proteins in this family are involved in the breakdown of extracellular matrix in normal physiological processes, such as tissue remodeling, as well as in disease processes, such as metastasis.	0.372926	2.082898
AAAT	solute carrier family 1 member 5 : High expression of AAAT correlates with metastasis and invasion and plays an important role in tumour cell growth.	0.080108	2.808409
WDFY3	WD repeat and FYVE domain containing 3 : This gene encodes a phosphatidylinositol 3-phosphate-binding protein that functions as a master conductor for aggregate clearance by autophagy. This protein shuttles from the nuclear membrane to colocalize with aggregated proteins, where it complexes with other autophagic components to achieve macroautophagy-mediated clearance of these aggregated proteins. However, it is not necessary for starvation-induced macroautophagy.	0.197735	2.067479
VEGF165R	This gene encodes one of two neuropilins, which contain specific protein domains which allow them to participate in several different types of signaling pathways that control cell migration. Neuropilins bind many ligands and various types of co-receptors; they affect cell survival, migration, and attraction. Some of the ligands and co-receptors bound by neuropilins are vascular endothelial growth factor (VEGF) and semaphorin family members.	0.089005	1.430977

Next, in order to get higher number of protein hits, we pooled the exosomal proteins from 2 samples and ran in Mass spec. As expected we got many more proteins by loading more sample (366 vs the 122 previously), but the high levels of BSA and a few other proteins as detected on the gel, resulted in a significant dynamic range issue to go after lower abundance proteins from the exosomes. In the table below and figure 4, some selected proteins which have been implicated in Prostate cancer such as ARIP4, 1433Z, BMP-1 and HOOK3 are downregulated and SLC2A1, RHOC, RAC1, MMP1 are upregulated in Ppim1 overexpressing cells. This comparative proteomic analysis could serve as a basis for studying the underlying mechanisms of Pim1 kinase regulation of prostate cancer through crosstalk by stromal cells.

Results from second analysis:

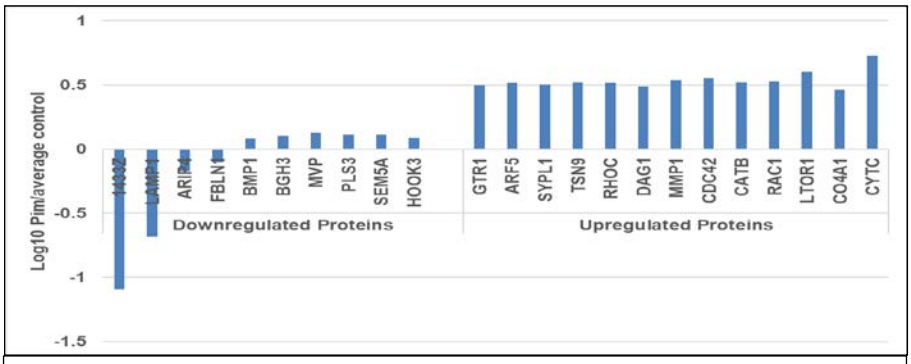
Downregulated proteins		Description	Log10 Pim/average control
1433Z	**14-3-3 protein zeta/delta**	The protein has been implicated in many cancers, including lung cancer, breast cancer, lymphoma, and head and neck cancer, through pathways such as mTOR, Akt, and glucose receptor trafficking. Notably, it has been associated with chemoresistance and, thus, is a promising therapeutic target for cancer treatment.	-1.094853751
LAMP1	**Lysosomal-associated membrane protein 1**	LAMP1 expression on the surface of tumor cells has been observed for a number of different cancer types, particularly in highly metastatic cancers	-0.681448428
ARIP4	**Androgen receptor-interacting protein 4**	DNA helicase that modulates androgen receptor (AR)-dependent transactivation in a promoter-dependent manner.	-0.170460751
FBLN1	**Fibulin 1**	Down regulated in most of the cancers, Fibulin 1 is a secreted glycoprotein that becomes incorporated into a fibrillar extracellular matrix. Calcium-binding is apparently required to mediate its binding to laminin and nidogen. It mediates platelet adhesion via binding fibrinogen.	-0.099417834
BMP1	**bone morphogenetic protein 1**	Loss of bone morphogenetic protein is associated with prostate cancer.	0.080634233
BGH3	**Transforming Growth Factor Beta Induced**	This gene encodes an RGD-containing protein that binds to type I, II and IV collagens. The RGD motif is found in many extracellular matrix proteins modulating cell adhesion and serves as a ligand recognition sequence for several integrins. This protein plays a role in cell-collagen interactions and may be involved in endochondrial bone formation in cartilage. The protein is induced by transforming growth factor-beta and acts to inhibit cell adhesion	0.103631873
MVP	**Major vault protein**	Required for normal vault structure. Vaults are multi-subunit structures that may act as scaffolds for proteins involved in signal transduction. Vaults may also play a role in nucleo-cytoplasmic transport. Down-regulates IFNG-mediated STAT1 signaling and subsequent activation of JAK. Down-regulates SRC activity and signaling through MAP kinases.	0.127755212
PLS3	**Plastin 3**	Plastins are a family of actin-binding proteins.Higher expression is found in breat cancer cells.	0.111443837
SEM5A	**Semaphorin 5A**	May promote angiogenesis by increasing endothelial cell proliferation and migration and inhibiting apoptosis.	0.114645551
HOOK3	**Hook microtubule-tethering protein 3**	HOOK3 is an adaptor protein for microtubule-dependent intracellular vesicle and protein trafficking. High-Level HOOK3 Expression Is an Independent Predictor of Poor Prognosis Associated with Genomic Instability in Prostate Cancer.	0.085634187

Upregulated Proteins		Description	Log10 Pim/average control
GTR1	**Solute Carrier Family 2 Member 1, Slc2A1**	SLC2A1 is expressed in breast cancer cells and is likely responsible for avid glucose uptake observed in established tumors.	0.496779212
ARF5	**ADP ribosylation factor 5**	play a role in vesicular trafficking and as activators of phospholipase D.	0.516700394
SYPL1	**Synaptophysin-like 1**	SYPL1 overexpression predicts poor prognosis of hepatocellular carcinoma and associates with epithelial-mesenchymal transition.	0.503239197
TSN9	**Tetraspanin-9**	The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility.	0.520730987
RHOC	**Ras homolog gene family, member C**	Overexpression of RhoC is associated with cell proliferation and causing tumors to become malignant. It has also been found to enhance the creation of angiogenic factors such as VEGF, which is necessary for a tumor to become malignant.	0.515580357
DAG1	**Dystroglycan**	The dystroglycan complex is involved in a number of processes including laminin and basement membrane assembly, sarcolemmal stability, cell survival, peripheral nerve myelination, nodal structure, cell migration, and epithelial polarization.	0.487696737
MMP1	**Matrix metalloproteinase-1**	MMPs are involved in the breakdown of extracellular matrix in normal physiological processes such as tissue remodeling, as well as in disease processes, such as metastasis.	0.537646122
CDC42	**Cell division control protein 42 homolog**	Activated Cdc42 activates by conformational changes p21-activated kinases PAK1 and PAK2, which in turn initiate actin reorganization and regulate cell adhesion, migration, and invasion.	0.551815364
CATB	**Cathepsin B**	Overexpression of the encoded protein has been associated with esophageal adenocarcinoma and other tumors.	0.520186274
RAC1	**Ras-related C3 botulinum toxin substrate 1**	Activating or gain-of-function mutations of Rac1 are shown to play active roles in promoting mesenchymal-type of cell movement assisted by NEDD9 and DOCK3 protein complex. Such abnormal cell motility may result in epithelial mesenchymal transition (EMT) – a driving mechanism for tumor metastasis as well as drug-resistant tumor relapse.	0.52862404
LTOR1	**Late Endosomal/Lysosomal Adaptor, MAPK And MTOR Activator 1**	LTOR1 is directly responsible for anchoring the Ragulator complex to membranes. Also required for late endosomes/lysosomes biogenesis it may regulate both the recycling of receptors through endosomes and the MAPK signaling pathway through recruitment of some of its components to late endosomes. May also play a role in RHOA activation.	0.603161464
CO4A1	**Collagen Type IV Alpha 1 Chain**	It functions as part of a heterotrimer and interacts with other extracellular matrix components such as perlecans, proteoglycans, and laminins.	0.46311414
CYTC	**Cytochrome C**		0.726349597

The proteins that were upregulated and downregulated by the induction of Pim in stromal cells are listed in this graph.

In conclusion, our results suggest that increasing Pim in stromal cells increases the number of exosomes that is being secreted from these stromal cells. It also shows that Pim induces increases in specific proteins. Many of these proteins are associated with rapid growth and increased motility including CDC42, RAC1, MMP1, RHOC and GTR1. Further investigations will be needed to see if these proteins impact on malignant epithelial cells.

Figure 4: **Comparative proteomic analysis of proteins secreted in the exosomes of Pim1 overexpressing BHPrS1 cells versus control cells. (Pooled samples)**



Proteomic analysis demonstrating proteins that were increased by Pim1 expression and those that were downregulated by this increase in exosomes.

Plans during the next reporting period

Task 1, Subtask 4,5 The MS/MS analysis of exosomes has been successful. However, to improve this result we will need to remove all albumin. We are hoping that we can use specific beads to get rid of albumin or cut the proteins from the gels prior to analysis.

Subtask 1, 3 , 5, 6 - Our ability to get samples from the operating room sterilely and to grow them in culture will allow us to evaluate CAF samples and compare them to stromal cell lines that contain Pim inducible proteins. Once these CAF samples are growing we should be able to use lentiviruses to transduce them with shPim1. During this year we have constructed this virus.

Task 2 – To examine task 2 in more detail we will be able to add Pim inhibitors to patient CAFs and examine the protein output in their exosomes using the technique that we have investigated above. We will also be able to compare them in terms of Gleason grade in terms of their response to inhibitors. In this task, the genetics of the patients may also play a role. We predict that patients with PTEN loss will have increased AKT activity and be less responsive to Pim inhibitors.

Task3- Dr. Singh has already begun to culture prostate organoids from humans that can be used in these studies. Using organoids from these patients can allow us to examine whether exosomes from CAFs will stimulate these malignant stem cells to grow. These cells are potentially an improvement over using BPH1 or PC3 cells that have been in culture for prolonged periods and also do not have the cancer stem cell phenotype that is found in organoids.

Training and professional development the project has provided.

This project has provided training for Dr. Neha Singh who was recruited specifically to carry out this work. She graduated with a BS in Chemistry from Banaras Hindu University, Varanasi, INDIA. She completed her Ph.D. in North Dakota State University and has been working on this project. Prior to joining this experimental team, she had not had knowledge of the issues related to the progression and outcomes of prostate cancer. In addition, she had not worked on myofibroblast/CAFs and studied their impact on cancer. This project has afforded her the opportunity to develop technical knowledge allowing her to isolate and interrogate exosomes from CAFs, investigate the protein makeup of exosomes, isolate and purify prostate stem cells, and examine the outcome of expression of Pim protein kinase in CAFs. This project has enabled her to learn about the Pim protein kinase as a target for prostate cancer therapy and to use in her research inhibitors of this enzyme obtained from industry that are being investigated in human clinical trials. As part of her training she attends Cancer Center grand rounds, Cancer Biology seminars and Therapeutic Development Conferences. She has interacted with invited speakers with expertise in exosomes. She attended the American Association of Cancer Research during the project period.

Dissemination of Results to the Community - *Nothing to Report*

Plans during the next reporting period to accomplish goals.

Impact
Impact on the development of the principle disciplines of this project – *Nothing to Report*
Impact on other disciplines – *Nothing to Report*
Impact on technology transfer –*Nothing to Report*
Impact on society beyond science and technology – *Nothing to Report*

Changes/problems
Changes in approach and reasons for change – *Nothing to Report*

Actual or anticipated problems or delays and actions or plans to resolve them.
To successfully be able to isolate CAFs from human prostate tumors it takes a strong Department of Urology and an active and attentive tumor bank. Since taking over the University of Arizona Cancer Center, im my position as Cancer Center Director, I have worked to enhance the activities of both of these entities.

In the previous year, we have had success in these endeavors. We have been able to get prostates from the operating room in a sterile condition. I have worked with the head of Urology, Dr. Lee, to make this possible. We are able to get three punch biopsies from the normal prostate and three from the cancer areas that are involved in the disease. These biopsies have been grown sterilely and will enable us to complete the analysis that we have designed in this proposal.

Changes that had a significant delay on actions or plans to resolve them. *Nothing to Report*

Changes in the use or care of human subjects, vertebrate animals, biohazards, and/or select change of agents. *Nothing to Report*

Products
Publications, conference papers, and presentations. *Nothing to Report*

Participants and other Collaborating Organizations

Name (Last, First)	Project Role	Nearest person month worked:	Contribution to Project	Funding Support
Kraft, Andrew	Principal Investigator	1	Oversees experimental design; provides scientific direction	NIH/NCI
Luevano, Libia	Research Specialist	3	Assisted fellow with all experiments discussed below	NIH/NCI
Singh, Neha	Postdoctoral Fellow	6	Performed exosome isolation, first and second proteomic analysis and comparisons between test and non-test cells	NIH/NCI

Change in the active other support of the PD/PIs or senior/key personnel since the last reporting period
Nothing to Report

Other organizations involved as partners.
Nothing New to Report.